Claims

1. A method for characterizing samples having fluorescent particles, comprising the steps of:

(a) monitoring intensity fluctuations of fluorescence emitted by the particles in at least one measurement volume by detecting sequences of photon counts by at least one photon detector,

(b) determining from the sequences of photon counts intermediate statistical data comprising at least two probability functions, $\hat{P}_1(\mathbf{n}_1),\hat{P}_2(\mathbf{n}_2),...$, of the number of photon counts, $\mathbf{n}_1,\mathbf{n}_2,...$, detected in different sets of counting time intervals,

(c) determining from said intermediate statistical data a distribution of particles as a function of at least two arguments, wherein one argument is a specific brightness of the particles, or a measure thereof, and another argument is a diffusion coefficient of the particles, or a measure thereof.

2. The method according to claim 1 wherein each set of counting time intervals consists of intervals of equal width while different probability functions $\hat{P}_{T_1}(\mathbf{n}_1),\hat{P}_{T_2}(\mathbf{n}_2),...$ correspond to counting time intervals of different widths $T_1,T_2,...$.

3. The method according claim 1 or 2 wherein in each set of counting time intervals these intervals are consecutive in time.

4. The method according to claim 1 or 2 wherein in each set of counting time intervals these intervals overlap.

5. A method according to any of claims 1 to 4 wherein said distribution function of particles is determined by fitting the experimentally determined probability functions

$\hat{P}_1(\mathbf{n}_1), \hat{P}_2(\mathbf{n}_2),...$ by corresponding theoretical probability functions $P_1(\mathbf{n}_1), P_2(\mathbf{n}_2),...$

6. A method according to any of claims 1 to 5 wherein said intermediate statistical data are processed applying inverse transformation with regularization and/or constraints.

7. A method according to any of claims 1 to 6 wherein the theoretical distributions $P_1(\mathbf{n}_1), P_2(\mathbf{n}_2),...$ are calculated through their generating functions $G_{P(n)}(\vec{\xi}) = \sum_{\mathbf{n}} \vec{\xi}^{\mathbf{n}} P(\mathbf{n})$.

8. A method according to any of the claims 1 to 7 wherein said distribution function of particles is determined by fitting the experimentally determined probability functions $\hat{P}_1(n_1), \hat{P}_2(n_2),...$ by corresponding theoretical probability functions $P_1(n_1), P_2(n_2),...$, which are preferably calculated through their generating functions $G_{P(n)}(\xi) = \sum_n \xi^n P(n)$.

9. A method according to any of the claims 1 to 8 wherein in calculations of the theoretical distributions $P_1(\mathbf{n}_1), P_2(\mathbf{n}_2),...$ the optical spatial brightness function $B(\vec{r})$ is accounted for by a separately determined relationship between brightness B and volume elements dV.

10. A method according to claim 9 wherein the relationship between the spatial brightness B and volume elements dV is expressed through a variable $x = \ln(B_0 / B)$ by a relationship $\frac{dV}{dx} = A_0(1 + a_1 x + a_2 x^2)x^{a_3}$, where B_0 is maximum brightness and A_0, a_1, a_2 and a_3 are empirical parameters of the spatial brightness function.

11. A method according to one of the claims 7 to 10 wherein the generating function is calculated using the expression $G(\xi) = \exp[\int dq c(q) \int d^3\mathbf{r}(e^{(\xi-1)\eta TB(\mathbf{r})} - 1)]$, where $c(q)$

is the density of particles with specific brightness q, T is the length of the counting time interval, and $B(\mathbf{r})$ is the spatial brightness profile as a function of coordinates.

12. A method according to claims 1 to 11 wherein concentrations of particles are selected to be approximately one or less molecules per measurement volume.

13. A method according to claims 1 to 12 wherein said photon detector is either an avalanche photodiode or a photomultiplier.

14. A method according to claims 1 to 13 wherein at least two photon detectors are used monitoring fluorescence of different wavelenghts or polarization.

15. A method according to any of the claims 1 to 14 wherein said fluorescent particles are characterized applying an homogeneous fluorescence assay.

16. A method according to any of the claims 1 to 15 for use in diagnostics, high throughput drug screening, optimization of properties of molecules and identification of specific cell populations.

17. Use of a confocal apparatus for performing the method according to any of the claims 1 to 16, said confocal apparatus preferably comprising:
a radiation source (12) for providing excitation radiation (14),
an objective (22) for focussing the excitation radiation (14) into a measurement volume (26),
a detector (42) for detecting emission radiation (30) that stems from the measurement volume (26), and
an opaque means (44) positioned in the pathway (32) of the emission radiation (30) or excitation radiation (14) for erasing the central part of the emission radiation (30) or excitation radiation (14).